

CLAIMS

That which is claimed is:

1. A method for detecting fusion of an enveloped retrovirus to a target cell, the method comprising:

contacting a target cell with an enveloped retroviral virion, the virion containing a chimeric viral protein comprising a reporter polypeptide operably joined to a viral accessory protein, wherein the reporter polypeptide provides a detectable signal upon intracellular delivery of the chimeric viral protein into the target cell, which detectable signal is not detectable prior to said intracellular delivery;

detecting the presence or absence of the detectable signal;

wherein the presence of the detectable signal indicates the virion has fused with the target cell.

2. The method of claim 1, wherein the enveloped retroviral virion is a human immunodeficiency virus (HIV) virion.

3. The method of claim 2, wherein the chimeric viral protein comprises beta-lactamase (BlaM) operably linked to Viral protein R (Vpr).

4. The method of claim 1, wherein the reporter polypeptide of the chimeric viral protein provides the detectable signal by cleaving a substrate in the target cell.

5. The method of claim 4, wherein the reporter polypeptide is beta-lactamase.

6. The method of claim 5, wherein the substrate is CCF2.

7. The method of claim 1, wherein the viral accessory protein of the chimeric viral protein is Viral protein R (Vpr).

8. The method of claim 1, wherein the reporter polypeptide is beta-lactamase (BlaM).
9. The method of claim 1, wherein the chimeric viral protein comprises beta-lactamase (BlaM) operably joined to Viral protein R (Vpr).
10. The method of claim 9, wherein BlaM and Vpr are joined through a spacer peptide.
11. The method of claim 1, wherein the retroviral virion is a pseudotyped virion, and wherein the envelope protein of the psuedotyped virion is not endogenous to the retroviral virion.
12. A method for detecting fusion of an human immunodeficiency virus (HIV) virion to a target cell, the method comprising:
 - contacting a target cell with an HIV virion containing a chimeric viral protein, wherein the chimeric viral protein comprises a beta-lactamase(BlaM) polypeptide operably linked to a viral accessory protein, and wherein the cell contains a BlaM substrate so that intracellular introduction of the chimeric viral protein into the target cell results in cleavage of the substrate by BlaM and production of a detectable signal;
 - wherein detection of the detectable signal indicates that the HIV virion has fused with the cell.
13. The method of claim 12, wherein the viral accessory protein of the chimeric viral protein is Viral protein R (Vpr).
14. The method of claim 13, wherein BlaM and Vpr are operably linked through a spacer peptide.
15. The method of claim 12, wherein the HIV virion is a pseudotyped HIV virion, and wherein the envelope protein of the psuedotyped virion is not endogenous to the HIV virion.

16. A method for identifying an agent that modulates fusion of a human immunodeficiency virus (HIV) virion to a target cell, the method comprising:
contacting a target cell with a candidate agent and with an HIV virion containing a chimeric viral protein, wherein the chimeric viral protein comprises a beta-lactamase(BlaM) polypeptide operably linked to a Viral protein R (Vpr) polypeptide, and wherein the cell contains a BlaM substrate so that intracellular introduction of the chimeric viral protein into the target cell results in cleavage of the substrate by BlaM and production of a detectable signal;
detecting the presence or absence of the detectable signal;
wherein detection of an increase or decrease in the detectable signal in the presence of the candidate agent compared to detectable signal in the absence of the candidate agent indicates that candidate agent modulates fusion of the HIV virion to the target cell.

17. The method of claim 16, wherein BlaM and Vpr are joined through a spacer peptide.

18. The method of claim 16, wherein the HIV virion is a pseudotyped HIV virion having an envelope protein that is not endogenous to the HIV virion.

19. The method of claim 16, wherein the envelope protein is an envelope protein of a virus other than HIV.

20. A method for identifying a viral envelope protein that facilitates viral fusion to a target cell, the method comprising:
contacting a target cell with a pseudotyped HIV virion having a non-endogenous viral envelope protein incorporated into the virion envelope, the pseudotyped HIV virion containing a chimeric viral protein comprising a beta-lactamase(BlaM) polypeptide operably linked to a Viral protein R (Vpr) polypeptide, and wherein the cell contains a BlaM substrate so that intracellular introduction of the chimeric viral protein into the target cell results in cleavage of the substrate by BlaM and production of a detectable signal;
detecting the presence or absence of the detectable signal;

wherein detection of the detectable signal indicates that the non-endogenous viral envelope protein facilitates viral fusion.

21. The method of claim 19, wherein BlaM and Vpr are operably linked through a spacer peptide.

22. The method of claim 19, wherein the non-endogenous viral protein is a naturally-occurring viral protein.

23. The method of claim 20, wherein the method further comprises contacting the target cell with a candidate agent, wherein detection of the detectable signal in the absence of the candidate agent and detection of a decrease in the detectable signal in the presence of the candidate agent indicates the candidate agent inhibits viral fusion facilitated by the non-endogenous viral envelope protein.

24. An isolated chimeric viral protein comprising, operably linked from N-terminus to C-terminus, a beta-lactamase (BlaM) polypeptide, a spacer peptide, and a Viral protein R (Vpr) polypeptide.

25. The chimeric viral protein according to claim 24, wherein the spacer peptide comprises at least six glycine residues.

26. An isolated polynucleotide sequence encoding the chimeric viral protein of claim 24.

27. An isolated vector comprising the polynucleotide of claims 26.

28. An isolated recombinant cell containing the polynucleotide of claim 26.

29. An isolated enveloped virion containing the chimeric viral protein of claim 24.

30. A kit for detecting fusion of an enveloped retroviral virion to a target cell, the kit comprising at least one of:

- the chimeric viral protein of claim 24;
- an isolated vector comprising a polynucleotide sequence encoding the chimeric viral protein;
- an isolated enveloped virion containing the chimeric viral protein.

31. The kit of claim 30, wherein the kit further comprises a substrate cleavable by BlaM, which substrate is suitable for loading into a target cell.